

WHAT IS CLAIMED IS:

1. An *in vitro* process for producing more than one copy of a specific nucleic acid, said process being independent of a requirement for the introduction of an intermediate structure for the production of said specific nucleic acid, said process comprising the steps of:

- (a) providing a nucleic acid sample containing or suspected of containing the sequence of said specific nucleic acid;
- (b) contacting said sample with a mixture comprising:
  - (i) nucleic acid precursors,
  - (ii) one or more specific nucleic acid primers each of which is complementary to a distinct sequence of said specific nucleic acid, and
  - (iii) an effective amount of a nucleic acid producing catalyst; and
- (c) allowing said mixture to react under isostatic conditions of temperature, buffer and ionic strength, thereby producing more than one copy of said specific nucleic acid.

2.    The process of claim 1 wherein said specific nucleic acid is single-stranded or double-stranded.
3.    The process of claim 1 wherein said specific nucleic acid is selected from deoxyribonucleic acid, ribonucleic acid, a DNA.RNA hybrid or a polymer capable of acting as a template for a nucleic acid polymerizing catalyst.
4.    The process of claim 1 wherein said specific nucleic acid is in solution.
5.    The process of claim 4 further comprising the step of treating said specific nucleic acid with a blunt-end promoting restriction enzyme.
6.    The process of claim 1 wherein said specific nucleic acid is isolated or purified prior to the contacting step (b) or the reacting step (c).
7.    The process of claim 6 wherein said isolation or purification of said specific nucleic acid is carried out by means of sandwich or sandwich capture.
8.    The process of claim 7 further comprising the step of releasing said captured specific nucleic acid.

9. The process of claim 8 wherein said releasing step is carried out by means of a restriction enzyme.

10. The process of claim 1 wherein said nucleic acid precursors are selected from nucleoside triphosphates and nucleoside trisphosphate analogs, or a combination thereof.

11. The process of claim 10 wherein said nucleoside triphosphates are selected from deoxyadenosine 5'-triphosphate, deoxyguanosine 5'-triphosphate, deoxythymidine 5'-triphosphate, deoxycytidine 5'-triphosphate, adenosine 5'-triphosphate, guanosine 5'-triphosphate, uridine 5'-triphosphate and cytidine 5'-triphosphate, or a combination of any of the foregoing.

12. The process of claim 10 wherein said nucleoside triphosphate analogs are naturally occurring or synthetic, or a combination thereof.

13. The process of claim 10 wherein at least one of said nucleoside triphosphates or nucleoside triphosphate analogs is modified on the sugar, phosphate or base.

14. The process of claim 1 wherein said specific nucleic acid primers are selected from deoxyribonucleic acid, ribonucleic acid, a DNA.RNA copolymer, or a polymer capable of hybridizing or forming a base-specific pairing complex and initiating nucleic acid polymerization.

15. The process of claim 1 wherein said specific nucleic acid primers comprise oligo- or polynucleotides.

16. The process of claim 1 wherein said specific nucleic acid primers contain a 3'-hydroxyl group or an isosteric configuration of heteroatoms.

17. The process of claim 16 wherein said heteroatoms are selected from nitrogen, sulfur, or both.

18. The process of claim 1 wherein said specific nucleic acid primers are not substantially complementary to one another.

19. The process of claim 18 wherein said specific nucleic acid primers contain no more than five complementary base-pairs in the sequences therein.

20. The process of claim 1 wherein said specific nucleic acid primers comprise from about 5 to about 100 nucleotides.

21. The process of claim 20 wherein said specific nucleic acid primers comprise from about 8 to about 20 nucleotides.

22. The process of claim 1 wherein said specific nucleic acid primers comprise at least one non-complementary nucleotide or nucleotide analog base, or at least one sequence thereof.

23. The process of claim 22 wherein said specific nucleic acid primers further comprise from about 1 to about 200 noncomplementary nucleotide or nucleotide analogs.

24. The process of claim 23 wherein said noncomplementary nucleotide or nucleotide analogs in said specific nucleic acid primers comprise from about 5 to about 20 nucleotides.

25. The process of claim 22 wherein said noncomplementary base sequence or sequences are linked together by other than a phosphodiester bond.

26. The process of claim 1 wherein said nucleic acid producing catalyst is selected from DNA polymerase and reverse transcriptase, or both.

27. The process of claim 1 wherein said nucleic precursors or said specific primers or both are modified by at least one intercalating agent.

28. The process of claim 1 further comprising the step (d) of detecting the product produced in step (c).

29. The process of claim 28 wherein said detecting step (d) is carried out by means of incorporating into the product a labeled primer, a labeled precursor, or a combination thereof.

30. The process of claim 1 further comprising the step of regenerating said one or more specific nucleic acid primers.

31. An *in vitro* process for producing more than one copy of a specific nucleic acid, said products being substantially free of any primer-coded sequences, said process comprising the steps of:

- (a) providing a nucleic acid sample containing or suspected of containing the sequence of said specific nucleic acid;
- (b) contacting said sample with a mixture comprising:
  - (i) nucleic acid precursors,
  - (ii) one or more specific polynucleotide primers comprising at least one ribonucleic acid segment each of which primer is substantially complementary to a distinct sequence of said specific nucleic acid, and
  - (iii) an effective amount of a nucleic acid producing catalyst; and
- (c) allowing said mixture to react under isostatic conditions of temperature, buffer and ionic strength, thereby producing at least one copy of said specific nucleic acid; and
- (d) removing substantially or all primer-coded sequences from the product produced in step (c) to regenerate a primer binding site, thereby allowing a new priming event to occur and producing more than one copy of said specific nucleic acid.

32. The process of claim 31 wherein said step (d) removing is carried by digestion with an enzyme.

33. The process of claim 32 wherein said enzyme comprises ribonuclease H.

34. The process of claim 31 wherein said nucleic acid precursors are modified or unmodified.

35. The process of claim 31 wherein said specific polynucleotide primers further comprise deoxyribonucleic acid.

36. The process of claim 31 wherein said specific polynucleotide primers contain a 3'-hydroxyl group or an isosteric configuration of heteroatoms.

37. The process of claim 36 wherein said heteroatoms are selected from nitrogen, sulfur, or both.

38. The process of claim 31 wherein said specific polynucleotide primers further comprise from about 1 to about 200 noncomplementary nucleotide or nucleotide analogs.



39. An *in vitro* process for producing more than one copy of a specific nucleic acid, said products being substantially free of any primer-coded sequences, said process comprising the steps of:

- (a) providing a nucleic acid sample containing or suspected of containing the sequence of said specific nucleic acid;
- (b) contacting said sample with a mixture comprising:
  - (i) unmodified nucleic acid precursors,
  - (ii) one or more specific chemically-modified primers each of which primer is substantially complementary to a distinct sequence of said specific nucleic acid, and
  - (iii) an effective amount of a nucleic acid producing catalyst; and
- (c) allowing said mixture to react under isostatic conditions of temperature, buffer and ionic strength, thereby producing at least one copy of said specific nucleic acid; and
- (d) removing substantially or all primer-coded sequences from the product produced in step (c) to regenerate a primer binding site, thereby allowing a new priming event to occur and producing more than one copy of said specific nucleic acid.

40. The process of claim 39 wherein said step (d) removing is carried by digestion with an enzyme.

41. The process of claim 40 wherein said enzyme comprises ribonuclease H.

42. The process of claim 39 wherein said specific chemically modified primers are selected from ribonucleic acid, deoxyribonucleic acid, a DNA.RNA copolymer, and a polymer capable of hybridizing or forming a base-specific pairing complex and initiating nucleic acid polymerization, or a combination of any of the foregoing.

43. The process of claim 39 wherein said specific chemically modified primers contain a 3'-hydroxyl group or an isosteric configuration of heteroatoms.

44. The process of claim 43 wherein said heteroatoms are selected from nitrogen, sulfur, or both.

45. The process of claim 39 wherein said specific chemically modified primers are selected from nucleoside triphosphates and nucleoside triphosphate analogs, or a combination thereof, wherein at least one of said nucleoside triphosphates or analogs is modified on the sugar, phosphate or base.

46. The process of claim 39 wherein said specific chemically modified primers further comprise from about 1 to about 200 noncomplementary nucleotide or nucleotide analogs.

47. An *in vitro* process for producing more than one copy of a specific nucleic acid, said products being substantially free of any primer-coded sequences, said process comprising the steps of:

- (a) providing a nucleic acid sample containing or suspected of containing the sequence of said specific nucleic acid;
- (b) contacting said sample with a mixture comprising:
  - (i) unmodified nucleic acid precursors,
  - (ii) one or more specific unmodified primers comprising at least one segment each of which primer comprises at least one non-complementary sequence to a distinct sequence of said specific nucleic acid, such that upon hybridization to said specific nucleic acid at least one loop structure is formed, and
  - (iii) an effective amount of a nucleic acid producing catalyst; and
- (c) allowing said mixture to react under isostatic conditions of temperature, buffer and ionic strength, thereby producing at least one copy of said specific nucleic acid; and
- (d) removing substantially or all primer-coded sequences from the product produced in step (c) to regenerate a primer binding site, thereby allowing a new priming event to occur and producing more than one copy of said specific nucleic acid.

48. The process of claim 47 wherein said step (d) removing is carried by digestion with an enzyme.

49. The process of claim 48 wherein said enzyme comprises ribonuclease H.

50. The process of claim 47 wherein said specific unmodified primers are selected from ribonucleic acid, deoxyribonucleic acid, a DNA.RNA copolymer, and a polymer capable of hybridizing or forming a base-specific pairing complex and initiating nucleic acid polymerization, or a combination of any of the foregoing.

51. The process of claim 47 wherein said specific unmodified primers further comprise from about 1 to about 200 noncomplementary nucleotide or nucleotide analogs.

52. A promoter-independent non-naturally occurring nucleic acid construct which when present in a cell produces a nucleic acid without the use of any gene product coded by said construct.

53. The construct of claim 52 comprising double-stranded and single-stranded nucleic acid regions.

54. The construct of claim 52 wherein said nucleic acid comprises deoxyribonucleic acid, ribonucleic acid, a DNA.RNA copolymer, or a polymer capable of hybridizing or forming a base-specific pairing complex and initiating nucleic acid polymerization.

55. The construct of claim 52 comprising at least one modified nucleotide or nucleotide analog.

56. The construct of claim 52 comprising at least one single-stranded region.

57. The construct of claim 56 wherein said single-stranded region comprises a bubble.

58. The construct of claim 57 wherein said bubble comprises at least one complementary sequence to a nucleic acid present in the cell.

59. The construct of claim 57 wherein said bubble comprises at least one polyT sequence.

60. A conjugate comprising a protein-nucleic acid construct, said nucleic acid construct not coding for said protein, and which conjugate produces a nucleic acid when present in a cell.

61. The conjugate of claim 60 wherein said protein comprises an RNA polymerase or a subunit thereof and the nucleic acid construct contains the corresponding RNA polymerase promoter.

62. The conjugate of claim 61 wherein said RNA polymerase is selected from T7, T3 and SP6, or a combination of any of the foregoing.

63. The conjugate of claim 60 wherein said protein comprises DNA polymerase or reverse transcriptase and said nucleic acid construct contains at least one sequence complementary to an RNA molecule.

64. The conjugate of claim 60 wherein said nucleic acid construct is double-stranded, single-stranded, or partially single-stranded.

65. The conjugate of claim 60 wherein said nucleic acid construct comprises at least one chemically modified nucleotide or nucleotide analog.

66. The conjugate of claim 60 wherein said protein is linked to said nucleic acid construct by means of a covalent linkage.

67. The conjugate of claim 60 wherein said protein is linked to said nucleic acid construct by means of base-pairing of complementary nucleic acid sequences.

68. The conjugate of claim 60 wherein said protein is linked to said nucleic acid construct by means of a nucleic acid binding protein.

69. The conjugate of claim 68 wherein said nucleic acid binding protein comprises a repressor protein bound to an enzyme.

70. The conjugate of claim 60 wherein said protein is linked to said nucleic acid construct by means of ligand receptor binding.

71. The conjugate of claim 60 wherein the nucleic acid produced is deoxyribonucleic acid, ribonucleic acid, or a combination thereof.

72. The conjugate of claim 60 wherein the nucleic acid produced is sense or antisense, or both.



73. An *in vivo* process for producing a specific nucleic acid, said process comprising the steps of:

(a) providing a conjugate comprising a protein-nucleic acid construct, said conjugate being capable of producing a nucleic acid when present in a cell; and

(b) introducing said conjugate into a cell, thereby producing said specific nucleic acid.

74. The process of claim 73 wherein said construct comprises at least one promoter.

75. The process of claim 73 wherein said construct comprises at least one complementary sequence to a primer present in the cell.

76. The process of claim 73 wherein said nucleic acid construct codes for the protein in said conjugate.

77. The process of claim 73 wherein said nucleic acid construct codes for a protein other than the protein in said conjugate.

78. The process of claim 77 wherein said other protein comprises a nucleic acid polymerase.

79. The process of claim 78 wherein said polymerase comprises an RNA polymerase and said nucleic acid construct comprises a promoter for said RNA polymerase.

80. The process of claim 78 wherein said polymerase comprises a DNA polymerase or reverse transcriptase.

81. A construct comprising a host promoter located on the construct such that the host transcribes a sequence in the construct coding for a different RNA polymerase which after translation is capable of recognizing its cognate promoter and transcribing from a DNA sequence of interest in the construct with said cognate promoter oriented such that it does not promote transcription from the construct of said different RNA polymerase.

82. The construct of claim 81 wherein said host promoter comprises a prokaryotic or eukaryotic promoter upstream from the host promoter.

83. The construct of claim 81 wherein said host promoter and the promoter for the second RNA polymerase are located on opposite strands.

84. The construct of claim 82 wherein said prokaryotic promoter comprises a RNA polymerase.

85. The construct of claim 82 wherein said eukaryotic promoter is selected from Pol I, Pol II and Pol III, or a combination of any of the foregoing.

86. The construct of claim 81 wherein said second RNA polymerase is selected from T7, T3 and SP6, or a combination of any of the foregoing.

87. The construct of claim 81 wherein said DNA sequence of interest comprises sense or antisense, or both.

88. The construct of claim 81 wherein said DNA sequence of interest comprises deoxyribonucleic acid or ribonucleic acid.

89. The construct of claim 81 wherein said DNA sequence of interest encodes a protein.

90. The construct of claim 81 comprising at least one chemically modified nucleotide.

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